COMMENTARY

UPDATED Reporting research antibody use: how to increase experimental reproducibility [v2; ref status: indexed, http://f1000r.es/1np]

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Abstract

Research antibodies are used in a wide range of bioscience disciplines, yet it is common to hear dissatisfaction amongst researchers with respect to their quality. Although blame is often attributed to the manufacturers, scientists are not doing all they can to help themselves. One example of this is in the reporting of research antibody use. Publications routinely lack key details, including the host species, code number and even the company who supplied the antibody. Authors also fail to demonstrate that validation of the antibodies has taken place. These omissions make it harder for reviewers to establish the likely reliability of the results and for researchers to reproduce the experiments. The scale of this problem, combined with high profile concerns about experimental reproducibility, has caused the Nature Publishing Group to include a section on antibody information in their recent Reporting Checklist for Life Science Articles. In this commentary we consider the issue of reporting research antibody use and ask what details authors should be including in their publications to improve experimental reproducibility.

Article Status Summary

Referee Responses

Referees	1	2	3
v1 published 10 Jul 2013	? report 1	✓ report 1	✓ report 1
v2 published 23 Aug 2013 UPDATED	✓ report		✓ report

1 **David Soll**, University of Iowa USA

2 **John Colyer**, University of Leeds UK

3 **Simon Glerup**, Aarhus University Denmark

Latest Comments

Andrew Chalmers, University of Bath, UK
06 Mar 2014 (V2)

Mark Livingstone, Institut Pasteur, France
25 Feb 2014 (V2)

Andrew Chalmers, University of Bath, UK
20 Sep 2013 (V2)

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Competing interests: ADC is a shareholder in CiteAb Ltd, which runs CiteAb the antibody search engine.

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UPDATED **Changes from Version 1**

In producing the second version of our manuscript we followed suggestions from the reviewers and included more information and references regarding antibody validation. In addition, more emphasis on problems with batch-batch variability, the importance of knowing the antigen and a reference which shows good antibody reporting was added. We also added more emphasis on the fact that change is likely to require help from journals, specifically that they include antibody reporting guidelines in their instructions to authors.

Antibody information is routinely omitted from publications

Neuroscience, cancer research, regenerative medicine, infection and immunity, cell biology and cardiovascular research are just some of the fields in which research antibodies are commonly used. The sheer scale of their use is illustrated by huge sales, estimated to be worth in excess of $1.6 billion annually[1]. Despite, or perhaps because of this widespread use, it is common to hear dissatisfaction among research scientists about the quality of these antibodies[2–4]. The finger of blame is often pointed at the manufacturers, yet it is questionable whether scientists themselves are doing everything they can to help the situation; surely not all problems can be placed at the door of the antibody manufacturer. One example of scientists not helping themselves is in their reporting of antibody use. There are many cases of good practice (For example[5]) and detailed reporting, but all too frequently authors omit key details. These include the host species and code numbers, but even the source of the antibody may be left out. This makes it harder for reviewers to establish how well characterised the antibodies are and thus how reliable the data presented are likely to be. It also makes it more difficult for other researchers to accurately reproduce experiments.

Failure to report key information is not a new problem[2,6], but recent developments have increased efforts to find a solution. In particular, experimental reproducibility has been thrust into the limelight by high profile cases. For example, a study of "landmark" cancer research papers found that scientific findings from only 11% of them could be repeated[7]. Taken at face value this is a shocking statistic and, in an attempt to try to improve experimental reproducibility, the Nature Publishing Group have recently introduced a reporting checklist for life science articles[8]. This checklist highlights research antibodies as a reagent type for which reporting could be improved. A key question is; what information to provide? In this commentary we consider what information authors should be including in their publications to help improve experimental reproducibility.

Key details for reporting antibody experiments

Publications need to report core information regarding the antibodies that were used. This should include the name of the antibody, the company/academic who supplied the antibody, the host species in which the antibody was raised and whether the antibody is monoclonal or polyclonal. In addition, the catalogue or clone number needs to be mentioned. The catalogue or clone number is commonly omitted from current publications, but is important as large antibody companies will often have multiple antibodies to the same target, a unique identifier is therefore essential to allow unambiguous identification of the antibody concerned. For this reason the first step in improving reporting should be to make it mandatory for authors to include core antibody information, including a code or clone number for the antibodies they use.

A second type of information that should be reported relates to experimental details. The application the antibody was used for is of central importance. This information is normally present, but it can be hard to extract if the antibody information is listed in a 'Materials' section and separated from descriptions of the techniques. Having the antibody data and application data closely linked would avoid potential confusion. Furthermore, if a study uses samples from more than one species then it is also important to clearly link which antibodies were used in which species.

There are other features that could also be reported which may be particularly relevant to certain studies. For example, the antibody batch number is rarely included in methods sections, but it is common to hear concern about variability between different antibody batches. This if often anecdotal, but there are some published examples[9,10]. This type of variability is likely to be a particular issue with polyclonal antibodies[2], but may affect monoclonal antibodies[11]. We encourage scientists to report cases in which variability has been found and in these examples include batch numbers. Reporting the final antibody concentration or dilution is another piece of information which can help other researchers, especially if optimisation was required during the study.

It has been proposed that scientists should know the antigen which was used to raise the antibody[3]. There are exceptions, for example where antibodies have been raised from a cell/tissue lysate and the antigen is unknown, but for most cases the antigen or at least its location within the protein should be known, as it may have implications for interpretation of the results. In cases where it is relevant to the study authors should be encouraged to report the antigen location. Finally, there will be details of particular importance for individual techniques, we focus on research antibodies, but studies reporting therapeutic use would be an example in which specific details such as purity and dose need to be reported.

Antibody validation

The Nature Publishing Group checklist, mentioned above, requires authors to demonstrate that every antibody used in their study has been validated for use in each of the specific experiments and species used. The experimental process of antibody validation is complex, with the most rigorous methods being comparison of wildtype vs a knockdown/knockout tissue and/or use of a second antibody to different epitope. The validation must also be carried out for each experimental setup as specificity in one application, or even fixative, does not mean an antibody will be specific in another. It is also the case that the details that should be reported to demonstrate validation will be different for each application. For more information on antibody validation we highly recommend the following publications[12–18].

Our focus is on how to report antibody validation, which can be achieved in a number of ways. If an antibody has not been previously validated for the specific combination of application and species used, then it should be mandatory that validation be carried out and reported. This can often be included as supplementary information.

If the antibody has previously been validated then one or more citations could be given to highlight the validation. Alternatively, the publication could reference the antibody validation profile from publicly available databases such as 1degreebio, Antibodypedia, CiteAb or pAbmAbs (A more extensive list of databases is available from Pivotal Scientific). Again it is important that antibody suppliers and codes are used in publications so that each antibody can be unambiguously identified and the degree of previous characterisation assessed. If new validation has been carried out then this could also be deposited in a public database and the database cited, instead of or in addition to putting the data in the supplementary information. Including information to show validation has occurred would help reviewers and other researchers accurately assess the results.

Change will require help from journals and reviewers

It seems likely that significant change is not going to occur unless journals take a lead and encourage it by adding antibody reporting guidelines to their instructions to authors. The success of this has been demonstrated by the Journal of Comparative Neurology which has had extensive guidelines in place since 2006 and the Journal of Visualised Experiments which requires a table of materials, including catalogue numbers for all the reagents used, to be reported. The Nature Publishing Group checklist should improve reporting in their journals and it is encouraging to see that following publication of version 1 of this manuscript F1000Research and PeerJ have added our proposed guidelines to their instructions for authors (described below). Once a journal has added guidelines it will be crucial that peer-reviewers are encouraged to evaluate the reporting and enforce the guidelines.

A simple format for reporting antibody information

Based on the points discussed above we would suggest that journals adopt, and researchers use, the format shown in Box 1.

This format is meant as a guide and could be adapted as required; for example details of batch number, dilution or epitope could be added where particularly important. This information could also be usefully presented in a table if allowed by the journal. Adoption of these reporting guidelines will not eliminate researchers' frustrations with antibodies, but should help improve experimental reproducibility and scientists' productivity, something we all seek. An additional benefit for authors who include this information is that well annotated publications are easier for antibody companies and antibody search engines to highlight in their databases. This inclusion is likely to increase the number of researchers who access their work and so potentially the impact of the study.

Box 1. Suggested format for reporting antibody information

Publications using commercial antibodies should report the supplier and the code number. Publications using academic antibodies should report the source laboratory and relevant reference. We recommend the following format;

"The following antibodies were used, Mouse anti-protein A monoclonal antibody (Company E, catalogue number #1000) was used for ELISA with human cells as validated in (figure X or reference Y or validation profile Z) and western blotting in mouse tissue as validated in (figure X or reference Y or validation profile Z).

Author contributions

ADC conceived the idea behind the commentary and produced a draft manuscript. All authors were involved in the revision of the draft manuscript, production of a revised version 2 and have agreed to the final content.

Competing interests

ADC is a shareholder in CiteAb Ltd, which runs CiteAb the antibody search engine.

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We thank everybody who commented on Version 1, in particular the reviewers for their helpful comments. We are also very grateful to Dr Mike Browning (PhosphoSolutions, USA) for suggesting some interesting references on antibody validation that are now included and Dr Leslie Boyer (University of Arizona) for her helpful comments regarding therapeutic antibodies.

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PubMed Abstract | Publisher Full Text

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PubMed Abstract | Publisher Full Text

Current Referee Status:

Referee Responses for Version 2

David Soll
University of Iowa, Iowa City, IA, USA

Approved: 01 October 2013

Referee Report: 01 October 2013
I am happy with the changes and I find the paper acceptable.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Simon Glerup
Department of Biomedicine, Aarhus University, Aarhus, Denmark

Approved: 09 September 2013

Referee Report: 09 September 2013
No further revision required.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Responses for Version 1

Simon Glerup
Department of Biomedicine, Aarhus University, Aarhus, Denmark

Approved: 01 August 2013

Referee Report: 01 August 2013
This commentary is much needed in the field of life science. It is well written and concise. Andrew Chalmer's group has contributed significantly to the use of research antibodies by creating CiteAb. When operating the CiteAb search engine, I imagine that they constantly run into problems with publications with poorly described use of research antibodies.

I have two minor suggestions:

1. In the Antibody Validation paragraph, a statement could be included in the methods section of a paper regarding if, where and under what name antibody validation information or reviews has been posted in publically available databases. This would increase the value and transparency of these databases.

2. Unlike the previous reviewer, I think it is fine to mention CiteAb in the paper. After all, even Nature Publishing Group is a highly commercial enterprise. However, I suggest that a table could be included listing the relevant databases including CiteAb, pAbmAbs, Biobrea, Antibodypedia, 1degreebio, Antibody-Advizer etc. In this regard, I regret that the Checklist from Nature Publishing Group only refers to sites in which they have a commercial interest (1degreebio and Antibodypedia). I hope that other publishing groups are not tempted to do the same.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

1 Comment

Author Response

Andrew Chalmers, University of Bath, UK
Posted: 03 Sep 2013

We thank Professor Glerup for his helpful comments and share his concern about the Nature Publishing Group guidelines. We explain our response to each one in turn below.

1. The fact that if no previous validation has occurred then it should be carried out and reported and/or submitted to a public database has been made clearer.

2. This is a good point and we agree it is important to give an overview of available databases to allow readers to choose the most appropriate. For this reason we were careful to mention a range in our first version. However, we feel it would not be appropriate for us to compile a table given our clear affiliation to one database, instead we provide a link to the most complete list of databases we are aware of.

Competing Interests: No competing interests were disclosed.

John Colyer
University of Leeds, Leeds, UK

Approved: 24 July 2013

Referee Report: 24 July 2013

The title and abstract are clear and appropriate. The article is timely and written clearly and accessibly.

- It could be improved further by providing references for papers that are examples “of good practice and detailed reporting”, which might serve as a template for others.
- The process of antibody validation is worthy of more extensive discussion, as the research community needs to develop a clear understanding of the most appropriate tests to be performed in each experimental system, and standards which should be attained for acceptance of the status of “validated”. This data should be provided in supplementary data, or by reference to previous supplementary data if the same reagents are used in a new study.
- The importance of batch number is made, but could be emphasized more.
- Finally, the critical role of peer-reviewers in evaluating and enforcing these standards is key. Some discussion of this would enhance the manuscript.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

1 Comment

Author Response

Andrew Chalmers, University of Bath, UK
Posted: 03 Sep 2013

We thank Professor Colyer for his positive and helpful comments and explain our response to each one in turn below.

- A good idea, we have now added an example reference that illustrates good reporting practice (Antibody information is routinely omitted from publications’ section). Journals which already encourage good practice have also been highlighted (‘Change will require help from journals and reviewers’).
- We completely agree and have increased the amount we cover on this topic, but not attempted a full review as we feel such a complex topic is beyond the scope of this comment article. We have added some additional citations for readers who require more information (Antibody Validation section).
- The fact that if no previous validation has occurred then it should be carried out and reported and/or submitted to a public database has been made clearer. The fact that previous validation can be cited has also been spelled out (Antibody Validation section).
- Additional emphasis has been added regarding the problem of batch to batch variability (‘Key details for reporting antibody experiments’ section).
- This has been added to the ‘Change will require help from journals and reviewers’ section.

Competing Interests: No competing interests were disclosed.

David Soll
University of Iowa, Iowa City, IA, USA

Approved with reservations: 16 July 2013

Referee Report: 16 July 2013
This commentary is timely and well written, but it could be shortened or tightened up a bit for the purpose of conciseness. It also should include a few points noted in this review. The major point is the problem that lack of information in publications involving research antibodies affects assessment and future use. The discussion could be more efficient in stating that if methods were reported in a previous referenced article, then referencing that article in a new publication is sufficient, unless there are nuances (i.e., new uses of the antibody). It should also be made clear that such information be mandatory when an antibody is used in a particular way for the first time.

There are also a few things the author may want to include:

1. Many antibodies work on a particular protein in a particular cell type without knowledge of the protein domain(s) found. In spite of that they may be of value, so you don't have to identify the sequence molecule.

2. Some antibodies identify native conformation and therefore are not on a peptide sequence per se. Such antibodies are not unusually performed on denatured proteins in western blots, but may work in nature gels.

3. Some antibodies have not been fully characterized beyond reference to the data sheet provided by the company or source if necessary.

4. If the authors of a paper refer to the company, and catalog name of the antibody, prior characterization can access.

5. Antibody validation should go in the supplementary data to a paper.

6. Do not cite CiteAb in your paper - it sounds like an ad.

But all in all, this is a reasonable commentary. It reinforces what many already are advocating. The title and abstract were fine.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

1 Comment

Author Response

Andrew Chalmers, University of Bath, UK
Posted: 03 Sep 2013

We thank Professor Soll for his positive review and helpful comments. We have now addressed them and explain our response to each one in turn below:

'The discussion could be more efficient in stating that if methods were reported in a previous referenced article....'

The fact that previous validation can be cited has now been spelled out more clearly (Antibody Validation section).

'...information be mandatory when an antibody is used in a particular way for the first time'

The fact that if no previous validation has occurred then validation should be carried out and reported and/or submitted to a public database has been made clearer (Antibody Validation section). These are two key points and we appreciate the fact you raised them.

Things we have now included to respond to the numbered points raised.

1. More discussion of the importance of knowing the antigen for an antibody has been added, in particular raising the point that for some antibodies the antigen is not known, for example when they are raised to a complex cell or tissue lysate (key details for reporting antibody experiments section).

2. This comment is relevant to the experimental validation of antibodies, we have increased the amount we cover on this topic but not attempted a full review as we feel such a complex topic is beyond the scope of this comment article. We have added some addition citations for readers who require more information (Antibody Validation section).

3. We have made it clearer when validation should be carried out and how it should be reported if no previous validation has taken place (Antibody Validation section).

4. We have now repeated the importance of including catalogue numbers in the antibody validation section.

5. This is now made clear (Antibody Validation section).

6. We think giving examples of available antibody databases will be useful to readers and were careful to mention more than one database, we have now added a link to a more extensive list. We have also removed the second reference to CiteAb which was in the final section.

Competing Interests: No competing interests were disclosed.

Article Comments

Comments for Version 2

Author Response

Andrew Chalmers, University of Bath, UK
Posted: 06 Mar 2014

Dear Mark,

That is an interesting observation; including catalogue codes is a big step forward in terms of being able to identify the reagents used, but I don't have any news on their approach to whether validation information is required. It sounds like your observations suggests not.

Competing Interests: No competing interests were disclosed.

Mark Livingstone, Institut Pasteur, France
Posted: 25 Feb 2014

I'm interested to know how the *Nature* checklist is being used today. Looking through the articles published, I find no evidence of antibody validation using knockout/knockdown cells and tissues. It seems they have settle for publishing catalogue numbers of antibodies, and maybe they don't require that " *Antibodies should have been profiled to determine their sensitivity, specificity and range of reactivity in the assay being considered".* Is there any news?

Competing Interests: No competing interests were disclosed.

Author Response

Andrew Chalmers, University of Bath, UK
Posted: 20 Sep 2013

An interesting recent paper quantifies the % of antibodies that can be identified from scientific publications and found only 46% could be identified, with only 27% using code numbers.
https://peerj.com/articles/148/

Competing Interests: No competing interests were disclosed.

Author Response

Andrew Chalmers, University of Bath, UK
Posted: 16 Sep 2013

Dear Professor Finger we thank you for your support for our article and interesting comments. We absolutely agree about the importance of the antigen. Regarding newly generated antibodies, the simplest way might be for batch numbers to correspond to each uniquely raised antibody sample and for authors to report batch numbers. We discussed both issues in the article but decided not to include them in our core guidelines, instead mentioning them as additional things which could/should be reported. We felt that keeping the core guidelines to a minimum would make it more likely that they would be adopted by journals that do not currently have any guidelines. However, we agree in an ideal world all journals would have more extensive guidelines, such as found in The Journal of Comparative Neurology.

Competing Interests: No competing interests were disclosed.

Thomas Finger, School of Medicine, Department of Cell and Developmental Biology, University Colorado Denver, USA
Posted: 07 Sep 2013

The point made by this article is very important and cannot be emphasized enough. Articles that report results using unspecified reagents (e.g. antisera) are simply not repeatable. A significant problem with the minimum standards suggested in this article is the omission of specification of the antigen employed to generate each particular antiserum and whether the product has been affinity purified. The selection of antigen is crucial for interpretation of results since the nature of the antigen will determine to a large extent the degree of cross-reactivity (both in terms of cross species and across proteins) of the product. While specification of a catalogue number and supplier will usually allow a reader to obtain this information for currently marketed antisera, once the company removes the antiserum from the market or even ends commercial activity, the antigen information is usually difficult or impossible to obtain. Accordingly, detailed information on the nature of the antigen should be included in all published reports. I encourage the authors to modify their guidelines to embrace this additional requirement.

Further, manufacturers should indicate, perhaps by a suffix to a catalogue number, if an antibody has been newly generated even if using the same antigen as previously. We have now had several experiences of ordering the same product number and having the new antiserum fail. Upon enquiry from the source, we are told that this is a new batch generated by immunizing a new set of animals. I would argue that this is no longer the same reagent and therefore should be assigned a different catalogue number. The new lot would need to be validated entirely as if a new product were being tested.

Competing Interests: Associate editor, J. Comp. Neurol.

Comments for Version 1

Mike Browning, PhosphoSolutions, USA
Posted: 12 Jul 2013

I would like to compliment the authors on their very informative and timely article. I also heartily endorse their “Format for Reporting Antibody Information”. A key feature of this recommendation is that authors report the catalog number of the antibody they use. This is a very important recommendation for authors, but in my opinion, this format is only useful if antibody vendors also implement certain standard practices. Obviously vendors must never substitute a new antibody source for an existing catalog number. Moreover it is especially important in polyclonal antibodies that vendors insure that all batches of the polyclonal come from the same pool of antisera and never from different bleeds from the same rabbit. If these two provisions are followed then many of the problems with batch to batch variation in antibodies can be eliminated.

Competing Interests: CEO and owner of PhosphoSolutions LLC a manufacturer of antibodies, especially phosphospecific antibodies